Exhibit 25

Modification of an Automated Method for Determining Plasma and Erythrocyte Cholinesterase Activity in Laboratory Animals*

Michael C Carakostas DVM, PhD and Monica A Landis MLT
EI du Pont de Nemours and Company Inc, Haskall Laboratory for Toxicology and Industrial Medicine, PO Box 50, Elkton Road, Newark, DE 19714

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ABSTRACT. By altering the analytical parameters on an automated analyzer, analytical precision for measuring cholinesterase (ChE) activity in hemolysates was markedly improved in samples from several species. Manual and automated spectrophotometric analyses of plasma and erythrocyte ChE activity were optimized for use in rats, mice and dogs. Replicate ChE analyses were performed on plasma samples and on hemolysates made from whole blood or packed erythrocytes to determine the precision of the manual ChE method and 4 modifications of the automated method. Large method-related differences in precision were observed for the erythrocyte assay, but not the plasma assay. The addition of a nonionic detergent to make hemolysates was beneficial in determining erythrocyte ChE activity in the rat, but not in the mouse or dog. Species specific temperature conversion factors were necessary for comparing results from methods using different analytical temperatures. Analysis of whole blood hemolysates provided similar or better precision for determining erythrocyte ChE activity compared to using hemolysates made from packed erythrocytes. Comparisons of erythrocyte ChE results obtained from assays with even minor methodological differences should be approached with caution because of the many analytical factors which can affect results.

Estimating the potential neurotoxicity of cholinesterase (ChE) inhibitors in safety assessment studies typically includes measuring plasma and erythrocyte ChE activities. Usually these studies are carried out on rats, mice or occasionally dogs. Interpreting the significance of decreased plasma or erythrocyte ChE activity has often been controversial (1). While much of the controversy has centered on the statistical and biological aspects of interpreting ChE results, the impact of analytical factors has not received as much attention. Cholinesterase activity has been measured by a number of methods over the years (2-4). However, the analysis of animal samples using modern automated procedures is not as simple as one might expect considering the literature available on ChE analysis. Analytically imprecise assays will be inaccurate predictors of toxicologically significant effects. Discussions concerning the most appropriate method of interpreting the toxicologic significance of decreased ChE activity are moot unless the precision of the analytical method is adequate.

Changes in procedure are sometimes required when the reagent kits used are originally designed for human samples. This is frequently the case with samples which are already an analytical challenge, eg hemolysates or tissue homogenates. Several commercially available kits are manufactured for the manual or automated analysis of ChE activity. Most are based on the original method of Ellman (3). Using reagent kits and automated analyzers provides a convenient and cost-effective way of obtaining results for most clinically relevant analytes. However, there is usually a paucity of information about the potential differences between the analysis of animal and human samples for most kits. Using a typical ChE reagent kit we obtained excellent precision on animal plasma samples. However, imprecise or unusable results were often obtained when the erythrocyte ChE activity was determined on animal samples. These results appeared to be caused by 2 problems. First, the presence of erythrocyte stroma, and perhaps hemoglobin, interfered with the spectrophotometric detection of the chromagen in the reaction mixture. Secondly, the reaction rate for the hemolysate occurred much more slowly than for plasma (0.7 mA/sec versus 1.3 mA/sec). Due to slow reaction rate, changes in the reaction absorbance were more difficult for the analyzer to measure. Many reactions were determined to be "nonlinear" by the analyzer (eg zero-order rate kinetics were not present). Therefore, we investigated modifications to the existing automated procedure that would provide better precision and fewer nonlinear reactions in rat, mouse and canine samples.

MATERIALS AND METHODS

Equipment and Reagents

Manual analyses were performed on a RESPONSE spectrophotometer (Ciba-Corning Diagnostics, Oberlin, OH). Automated analyses were performed on an ENCORE II centrifugal analyzer (Serono Baker Diagnostics, Allentown, PA). A commercially available kit (Cholinesterase ReagentSet, Boehringer Mannheim Diagnostics, Indianapolis, IN) based on the Ellman method was used for both the manual and automated analyses (3). Nonionic detergent (Triton X-100, Sigma Chemical, St Louis, MO) and phosphate buffered saline (0.1M KH_2PO_4, pH 8; EM Science, Cherry Hill, NJ) were used to make some hemolysates.

Animals

Blood samples for analysis were obtained from young beagle dogs (Marshall Research Animals, North Rose, NY), adult Crl:CD BR rats (Charles River Laboratories Inc, Kingston, NY) and adult Crl:CD-1(ICR)BR mice (Charles River Laboratories Inc, Raleigh,

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NC). Blood was obtained from the jugular vein of dogs, and the orbital plexus of rats and mice while they were under light carbon dioxide anesthesia. For comparison purposes, blood samples from a limited number of human volunteers in our laboratory were also analyzed for ChE activity. While there was some attempt to maintain an equal number of animals and replicates in each comparison, the availability of animals and the time required to perform replicates of manual methods limited the value of "n" in some comparisons.

Analytical Procedures

Analyses for ChE activity were performed on plasma, whole blood hemolysate, and hemolysate from washed and unwashed packed erythrocytes. Unless otherwise specified, hemolysates were prepared by diluting whole blood 1:10 or packed erythrocytes 1:20 with dionized water or a 0.5% solution of nonionic detergent in buffer. Erythrocyte ChE activity (RBC ChE) was calculated from the whole blood (WB ChE) and plasma ChE (Pl ChE) results using a formula provided by the reagent manufacturer:

RBC ChE=WB ChE-[Pl ChE x (1-Hct)]/Hct

where Hct is the whole blood hematocrit. The units for reporting all RBC ChE results are international units/liter of packed erythrocytes (IU/L).

Manual analyses were performed according to the reagent manufacturer's instructions. For comparison purposes, this method was cosidered the reference method. Automated analyses were performed according to recommendations from the instrument manufacturer (M1) and 3 other modifications of that method (M2, M3, M4) (Table 1). Briefly, these modifications involved changes in the incubation time (T_i), total reaction time (T_f), the time "window" in which absorbance readings were taken (T_w), and the interval of time between specific absorbance readings used to determine the reaction rate (T_r). The instrument took absorbance readings every 2 sec throughout each T_w, but any interval of time in multiples of 2 seconds (T_r) could be selected for determining the reaction rate. Since the reaction rate for determining ChE activity was slow, several longer T_r periods were chosen for investigation. The values for T_f and T_w were not critical, but were lengthened to accomodate the longer T_r periods. The best T_i time was determined from a graphic printout from the analyzer of the reaction rate versus time. The analyses were performed at 30 C with a linearity limit of 0.02 absorbance units/sec. The instrument automatically rejected results from reactions in which the change in absorbance did not occur at a constant rate throughout any T_w period (nonlinear reactions). Also, reactions which were too slow to detect, had an absorbance reading exceeding a maximum limit (2.3 A), or which showed premature substrate exhaustion were automatically rejected. Results from cuvettes with rejected reactions were not reported by the analyzer. This assured that absorbance readings used to calculate each sample's ChE activity were from reactions with zero-order kinetics. (In a state of zero-order kinetics, the reaction rate was directly proportional to the concentration of enzyme [5].)

Table 1.
Instrument Parameters For Determining Cholinesterase Activity On An Encore II Analyzer By Four Methods (M1 - M4)

Parameters*	METHODS M1	M2	M3	M4
	(seconds)			
T_i	60	60	40	70
T_w	30	120	100	120
T_r	10	30	25	40
T_f	180	275	300	330

* T_i = "initial time", T_w = "window time", T_r = "rate time" T_f = total reaction time. See text for details.

The precision of each automated method was determined on 6 rat, mouse and human blood samples and 3 canine samples by repeatedly analyzing each sample. Each plasma sample was analyzed 6 times and each hemolysate was analyzed 10 times by each automated method. The precision of the manual method was determined on the same samples, but plasma samples were analyzed twice and hemolysates 4 times each. The mean Pl ChE result, from each respective method, and a single hematocrit result were used in the calculation of RBC ChE activity from WB ChE results. Hemolysates made from whole blood and packed erythrocytes were analyzed by each method. Coefficients of variation (CV) for each species by each method were calculated by averaging the individual CV's from each sample. Additional rat samples with a wide variety of ChE activities were analyzed using the manual method (considered a reference method) and M3. Similar comparisons between M2 and M3 using whole blood hemolysates and between hemolysates made with whole blood and packed erythrocytes (using M3) were also performed. Method comparisons were made using regression analysis (6).

Species specific temperature conversion factors were determined for rats and dogs from analyses performed at 25, 30 and 37 C. Conversion factors for rats were based on results from 20 plasma samples. Conversion factors for dogs were based on duplicate analyses of plasma samples from 3 dogs. Conversion factors for human samples were confirmed using 2 human-based serum controls and 3 plasma samples, each analyzed in duplicate.

The effect of using a nonionic detergent as a hemolyzing agent was determined using rat, dog and mouse samples (10 samples each, 5 from males and 5 from females). The nonionic detergent was diluted in phosphate buffer at concentrations of 0.05, 0.1, 0.5, 1.0 and 10%. Whole blood hemolysates were made using each concentration of nonionic detergent and were then analyzed for ChE activity. Enhancement or inhibition of ChE activity was determined by comparing each result to the ChE activity of a hemolysate from the same sample made with deionized water.

To investigate whether the hemolysate reaction rate could be increased by increasing the concentration of enzyme in the hemolysates, dilutions of a previously analyzed rat hemolysate in commercial control sera (Precinorm E, Precipath E, Boehringer Mannheim Gmblt, Mannheim, Germany) were made and analyzed. Various mixtures of control sera and rat blood hemolysate with hemoglobin concentrations of 0.2 g/dL to 2.25 g/dL were analyzed for ChE activity. A ChE result was also calculated for each mixture based on the relative amount of hemolysate and control sera in each mixture and their known activities. The effect of increasing the hemoglobin concentration on the analysis of hemolysate ChE activity was determined by comparing the calculated result with the result obtained by analyzing the mixture.

The ChE activities of hemolysates made from washed and unwashed packed erythrocytes were compared to determine the need for washing erythroctyes prior to analysis. Unwashed packed erythrocytes were obtained by pipetting erythrocytes directly from the bottom of a tube of centrifuged rat or dog blood (2000 x g for 10 min). Washed packed erythrocytes were obtained from the same samples using 2 washes of phosphate buffered saline. Three samples from each species were analyzed in triplicate using either washed or unwashed erythrocytes. A 2-way analysis of variance (species and washing) was used to determine if washing the erythrocytes prior to analysis was necessary.

RESULTS

The instrument parameter changes made in the modified methods resulted in marked improvements in erythrocyte ChE assay precision (Table 2). All methods provided good precision for the plasma assay. Longer T_r periods provided more precise (repeatable) results than shorter T_r periods. The decreased T_i in M3 seemed to increase precision for rat and mouse samples much more than for the dog samples, as the reaction in the dog seemed to develop much more slowly. A longer lag period (T_i) and T_r in M4 gave an increase in precision for canine samples not observed in the rat or mouse.

Table 2.
Precision Estimates (Coefficient of Variation, C.V.) For Five Methods Of Determining Cholinesterase Activity In Plasma And Erythrocytes, A Manual Method, An Automated Method (M1), And Three Modifications of the Automated Method (M2,M3,M4).

Species	Plasma	Calculated[a] RBC	Direct[b] RBC
	COEFFICIENTS OF VARIATION		
RAT (n=6)			
Manual	2.9	4.6	3.0
M1	3.2	19.0	14.5
M2	1.5	9.7	10.0
M3	0.4	1.4	1.8
M4	0.4	1.2	1.8
DOG (n=3)			
Manual	3.8	9.2	5.4
M1	0.3	21.3	14.5
M2	0.6	6.2	5.3
M3	0.2	5.7	7.2
M4	0.3	3.2	2.8
MOUSE (n=6)			
Manual	1.7	10.7	5.6
M1	0.6	15.2	18.4
M2	0.6	8.6	22.6
M3	0.4	2.4	3.8
M4	0.4	2.2	5.4
Human (n=6)			
Manual	2.1	5.0	3.5
M1	0.4	5.9	6.3
M2	0.9	3.2	3.7
M3	0.4	1.8	3.4
M4	0.5	0.9	1.1

[a] Cholinesterase activity calculated from whole blood and plasma results, see text for details of calculations.

[b] Cholinesterase activity determined from packed erythrocytes.

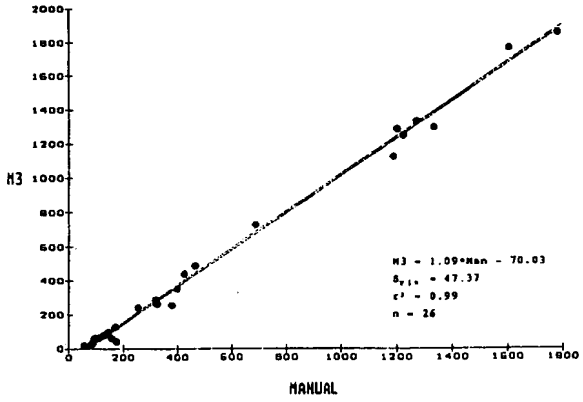

Figure 1. Regression analysis comparing plasma cholinesterase activity results from a manual method (Man) with results from a modified automated method (M3) using rat samples.

A comparison of the manual method and M3 is shown for rat plasma and erythrocyte ChE results in Fig 1 and 2, respectively. Plasma results from the manual method and M3 agreed quite closely. Similar findings were obtained for plasma ChE analyses when other combinations of methods were compared (results not shown). While there is general agreement between the 2 methods for erythrocyte activity at all levels tested, the results show substantial constant (y-intercept), random ($S_{y|x}$) and proportional (slope) differences. The y-intercept and slope were both significantly different from 0 and 1, respectively ($p<0.05$). A comparison of 2 automated meth-

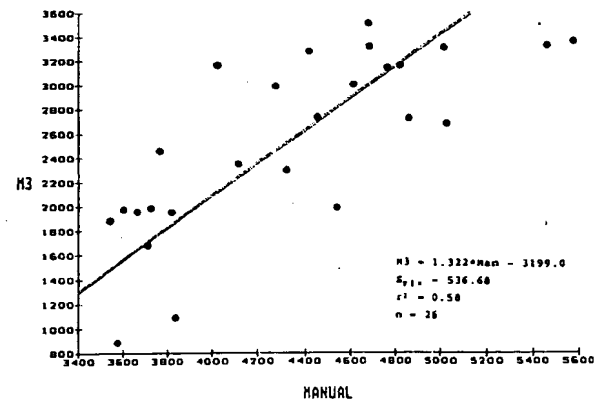

Figure 2. Regression analysis comparing erythrocyte cholinesterase activity results from a manual method (Man) with results from a modified automated method (M3) using rat samples (calculated erythrocyte method).

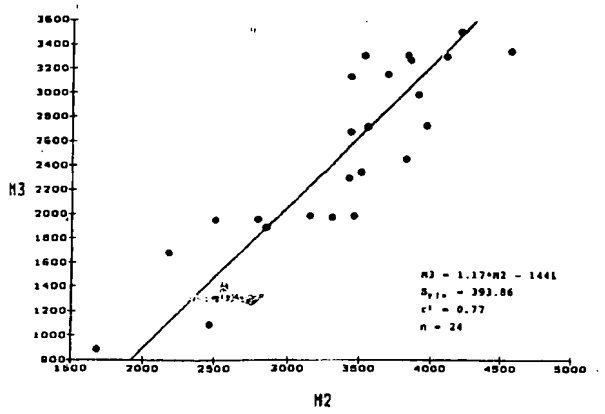

Figure 3. Regression analysis comparing rat erythrocyte cholinesterase activity results from two modified automated methods (M2 and M3).

ods (n=26) gave regression statistics of M3= 0.98*M2 + 6.87, $S_{y|x}$=21.47, r^2=0.99 for plasma. The comparison of M3 and M2 for RBC ChE is presented in Fig 3. Even in 2 methods as closely related as M2 and M3, the erythrocyte comparison showed substantial method-related differences, ie the intercept and slope were significantly different from 0 and 1, respectively ($p<0.05$).

Temperature conversion factors for human samples provided by the reagent manufacturer were confirmed in this study. However, these factors were substantially different from those established for the rat and dog (Table 3).

Concentrations of nonionic detergent up to 0.5% increased the ChE activity of rat hemolysates (Fig 4A). Concentrations greater than 0.5% had little additional effect. The presence of a detergent did not affect the mean activity of canine samples (Fig 4B). However, increased detergent concentrations had a variable effect on samples from different dogs, which markedly increased the standard deviation of the mean results. The presence of nonionic detergent increased ChE activity in some hemolysates and decreased it in others.

Table 3.
Species Specific Temperature Conversion Factors

	HUMAN[a]		RAT[b]	DOG[c]
Temperature	(A)	(B)		
25 C	1.00	1.00	1.00	1.00
30 C	0.78	0.77	0.86	0.85
37 C	0.60	0.58	0.70	0.70

[a] (A)= Manufacturers' stated factors
(B)= Factors established in our laboratory (n=5 samples run in duplicate)
[b] = n=20
[c] = n=3 samples run in duplicate

The presence of the detergent decreased the hemolysate activity in mice, particularly in female mice (Fig 4C). When the low whole blood result was used to calculate the erythrocyte activity an apparent decrease in the erythrocyte activity was observed.

Increasing the hemoglobin concentration in

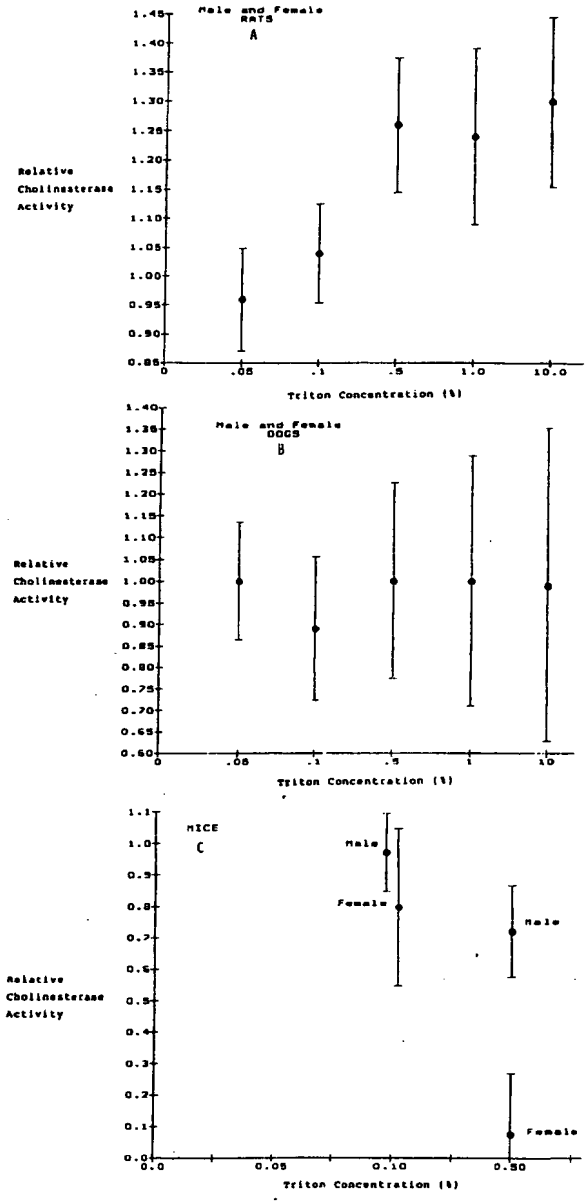

Figure 4A-C. Comparison of various concentrations of a nonionic detergent (Triton X-100) with water on the cholinesterase activity of whole blood hemolysates from rats (A), dogs (B), and mice (C). N=10; the same samples from 5 males and 5 females were analyzed for each Triton concentration. The abscissa of each figure represents the relative cholinesterase activity in a hemolysate made with Triton compared to the cholinesterase activity in a hemolysate from the same sample made with deionized water (Triton hemolysate activity/water hemolysate activity).

the control sera/hemolysate mixtures up to a hemoglobin concentration of 1.5 g/dL did not affect the measurement of ChE activity (Table 4). Hemoglobin concentrations of 2.25 g/dL and higher interfered with the spectrophotometric detection of the chromagen at 405 nm.

A comparison of erythrocyte ChE results calculated from packed erythrocyte and whole blood hemolysates is shown in Table 2. The comparison of ChE results for a variety of rat samples (n=26) analyzed using both whole blood (WB) and packed cells (PC) gave regression statistics of $PC=1.14*WB + 131.0$, $S_{y|x}= 279.34$, $r^2=0.86$. The intercept and slope were significantly different from 0 and 1, respectively ($p<0.05$). These results suggest that RBC ChE results from whole blood and packed erythrocyte hemolysates made from the same sample will have equivalent, but probably not equal, results.

Washing erythrocytes prior to determining ChE analysis to remove residual plasma did not appear necessary. The analysis of variance did not show any significant differences between results from washed and unwashed samples ($p=0.13$). Mean hemolysate ChE activity results (IU/L) from 3 canine samples, each analyzed in triplicate, were 84, 84 and 96 for unwashed cells, compared to 83, 78, and 102, respectively, for the washed cells. Mean hemolysate ChE activity results (IU/L) from 3 rat samples, each analyzed in triplicate, were 144, 156, and 152 for the unwashed cells, compared to 131, 153, and 138, respectively, for the washed cells.

DISCUSSION

The changes in the analytical parameters we investigated should be applicable to many automated analyzers. These changes allowed a more precise analysis of hemolysate ChE activity in spite of a slow reaction rate. The slow reaction rate and membrane stroma appeared to be primarily responsible for the imprecision observed in measuring erythrocyte ChE activity. The dilution required to hemolyze erythrocytes also reduced the ChE activity, slowing the analytical reaction rate. Attempts to increase the amount of activity by increasing the concentrations of enzyme in the hemolysate were limited by the interference of hemoglobin at concentrations above approximately 1.5 g/dL. The precision estimates from human samples were often much better than those from animal samples, regardless of the method used (see CV's in Table 2). Whatever the reason, animal samples were more difficult to analyze than human samples. Therefore, it appeared species specific changes in methodology were required to obtain adequate precision for determining erythrocyte ChE activity.

Alterations made in the analytical parameters resulted in 1 basic change. By using a longer time period (T_r) for measuring the increase in absorbance, a larger absorbance change was used to determine ChE activity in each sample. Since the interfering sources of variation appeared to be random rather than additive, their impact on measuring absorbance changes became relatively smaller. The changes in T_w and T_f were only made to accomodate the longer T_r period. The T_i time was selected so that absorbance readings from the initial period where reaction kinetics are not yet in equilibrium (lag phase) were disregarded. A graphic print-out of the change in absorbance over time was used to determine the best T_i. The instrument automatically disregarded readings from additional portions of the reaction not conforming to zero-order kinetics (ie a nonlinear change in the reaction rate).

The manual ChE method was considered the "reference" method in this study because it was the method provided by the reagent kit manufacturer. The comparison of the manual method with M3 (our current method of choice for rats and mice) showed a linear relationship existed between results from each method for both plasma and RBC ChE activity. However, unlike the plasma activity results, differences between the methods were quite marked in the erythrocyte assay (as indicated by the intercept and slope estimates in Fig 2). Random differences (estimated by $S_{y|x}$) might be accounted for by the imprecision present in the manual method. However, the large y-intercept and slope estimates in the RBC ChE comparisons suggested large method-related differences exist. Since the only difference between the plasma and erythrocyte assays for each method was the sample, it appeared that each method reacted differently to the presence of interfering substances in the hemolysates. Regression results from the comparison of RBC ChE results from 2 closely related automated methods, M2 and M3, supported this conclusion (Fig 3). While comparisons of plasma ChE results from different spectrophotometric methods might be possible, similar comparisons of erythrocyte results should be approached with caution, even if the methods appear quite closely related. These findings present a strong argument for converting ChE results into a standardized format (eg a percent of the control group mean value) for safety assessment studies.

Table 4.
Cholinesterase Activity Of Two Control Sera Containing Various Amounts of Rat Whole Blood Hemolysate. (Comparison Of "Actual" Results with "Expected" Results Calculated From Known Activity Of Both Samples.

	HEMOGLOBIN CONCENTRATION (gm/dl)	CHOLINESTERASE ACTIVITY (IU/L) ACTUAL	EXPECTED
Control Sera I	0.075	304	287
	0.150	312	297
	0.375	328	325
	0.750	396	372
	1.500	478	467
	2.250	NR	562
Control Sera II	0.075	123	113
	0.150	130	123
	0.375	150	151
	0.750	203	198
	1.500	288	293
	2.250	NR	388

NR - No Results. Results "flagged", absorbance exceeded 2.3A limit. Result not valid.

The most desirable enzyme assay is usually the one with the best precision. In our study this appeared to be M3, except in the dog where M4 was better. Method 3 was selected over M4 in rats and mice because it used shorter parameter times and had nearly the same precision as M4. Since the CV's in Table 2 were calculated from replicate analyses of samples from animals of different ages and sexes, only the variances of the replicates were deemed appropriate for combination into a mean estimate of method precision. Mean ChE results would not be very meaningful as reference values. The mean values of the rat samples shown in Table 5 are presented only to demonstrate the marked method-related differences in the erythrocyte results. The mouse and dog results showed a similar pattern. We selected M3 for the rat and mouse and M4 for the dog based on their superior precision, not because the results they provided matched those obtained using the reagent manufacturer's manual method. In toxicology studies where data from a control group are compared clinically and statistically to one or more treatment groups, it is important to reduce non-treatment related variance. The presence of significant variance unrelated to treatment may result in erroneous conclusions about the effect of a given material. Method-related variance in currently available data may be a significant contributor to the uncertainty over what constitutes a biologically significant decrease in ChE activity.

Table 5.
Mean Plasma And Erythrocyte (RBC) Cholinesterase Activity (U/L) Results Obtained From Rat Samples Using A Manual Method, An Automated Method (M1) And Three Modifications Of The Automated Method (M2,M3,M4)

	Plasma	Calculated[a] RBC	Direct[b] RBC
RAT (n=6)			
Manual	799[c]	3734	3307
M1	766	5710	4880
M2	800	3728	3323
M3	806	3004	2607
M4	807	2710	2357

[a] Cholinesterase activity calculated from whole blood and plasma results, see text for details of calculations.

[b] Cholinesterase activity determined from packed erythrocytes.

[c] Values are mean results from replicate analyses performed on a sample from each subject (n=6). See text for details of replicate analyses.

Because measured enzyme activity is dependent on the assay temperature, temperature conversion factors are often provided by reagent manufacturers. Manual assays are often more conveniently performed at 25 C, while clinical analyzers will perform the assays at 30 or 37 C. Enzyme activity results obtained from assays performed at different temperatures must be converted to the same temperature before they can be compared. We have noted previously that conversion factors provided by manufacturers are established for human samples and may not be accurate for animal samples (7). The conversion factors provided with the cholinesterase reagent kit used in this study were accurate for human samples, but not for rats or dogs. The new factors we have established are not method-specific and can be used for the conversion of ChE results from any spectrophotometric ChE assay.

A 0.5% solution of Triton X-100 was considered optimal for hemolyzing rat erythrocytes. Inhibited ChE activity in mouse hemolysates and a variable response in canine hemolysates made deionized water a better hemolyzing agent in these species. This finding in dogs conflicts with the results of a previous study which did not report any problems with a 5% solution of the same detergent used in this study (8). The problem can be avoided if packed erythrocytes are used, since it was discovered that it was the plasma ChE activity that was inhibited by the detergent in the whole blood hemolysates of mice and some dogs. While centrifugation of hemolysates is used by some laboratories to reduce the amount of stromal interference in hemolysates, this procedure intentionally removes a portion of the analyte being measured. We felt a better solution to the problem of stromal interference was to change the assay to accommodate the sample. By changing the way the analyzer measured ChE activity, we markedly increased precision without having to remove a portion of the analyte from each sample using a procedure which had the potential of markedly reducing analytical precision.

Using packed erythrocytes rather than whole blood to make hemolysates appeared a sensible way of eliminating several sources of variation. Neither the plasma ChE activity nor the hematocrit are required; this eliminates 2 potential sources of measurement error. Packed erythrocytes are particularly easy to use since we have shown that washing the cells is an unnecessary step (see Results). Washing the erythrocytes is time consuming, labor intensive, and impractical for large sample loads unless automated. Washing may also enhance reactivation of ChE inhibited by carbamates, making it impractical for safety assessment studies with these compounds. However, our results show that the whole blood method of determining erythrocyte ChE activity using the improved methodologies (M3 or M4) provided similar or better precision when compared to the packed cell method (Table 2). In spite of the seemingly odd calculation of erythrocyte activity required in the whole blood method, it provides pre-

cise results. We have continued to use the whole blood method for samples from all species. However, results from 2 hemolysates (1 from whole blood and 1 from packed erythrocytes) made from the same blood sample should give biologically equivalent, but probably not equal, results.

Inhibitors of plasma ChEs (butyrylcholinesterase) have been used in whole blood systems to specifically measure erythrocyte ChE activity without the need for calculations or blood separation (9). This may work in samples from humans, but plasma from animals often contain acetylcholinesterase and other choline esterases which may not be inhibited by a specific inhibitor of butyrylcholinesterase (9,10). The complexity introduced into ChE analysis by the addition of inhibitors with unknown activity seems unwarranted in light of the precision which can be obtained with either the calculated or direct RBC methods investigated in this paper.

Exposure concentrations in safety assessment studies on ChE inhibitors are often selected so that the only apparent treatment-related effect will be a decreased ChE activity. This places emphasis on a single clinical biochemical result that is usually not present in most safety assessment studies. Factors affecting the measurement of ChE activity in blood or tissues unrelated to the test material can have a marked impact on the outcome of the study, and hence the regulatory decisions on the use of the compound. There are many methods of measuring ChE activity in blood and tissue. Some have potentially less method-related variability than many current spectrophotometric methods. However, most of these methods are not practical in a busy clinical laboratory performing tests in support of a wide variety of safety assessment studies. Therefore the spectrophotometric method used in this study is likely to be in common use for some time. This method is capable of providing results on animal specimens with adequate precision, if appropriately adapted to the specific analyzer and species. Additional care is required in the interpretation of RBC ChE results, particularly historical data or a comparison of results from several laboratories, as RBC ChE results may be markedly affected by seemingly minor methodological differences.

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HOW YOU CAN REDUCE STRESS

Try these tips to cope with stressful situations:
Schedule anticipated stress. When possible, space stressful situations so they don't come all at once.
Arrange for privacy. Find a place and schedule a time where you can think alone without interruption.
Maintain control. Don't allow insignificant events to control you.
Establish support systems among family, friends and colleagues.
Don't procrastinate. Stressful situations get worse the longer you tolerate them.
Make decisions based on your needs rather than what others expect your needs to be.

The Newslink, Warwick Insurance Company, Four Gatehall Drive, PO Box 520, Morris Plains, NJ 07950

OVERCOMING CONVENTIONAL WISDOM WAS NEVER EASY...

For centuries, people believed that Aristotle was right when he said that the heavier an object, the faster it would fall to earth. Aristotle was regardedas the greatest thinker of all times and surely he could not be wrong. All it would have taken was for one brave person to take two objects, one heavy and one light, and drop them from a great height to see whether or not the heavier object landed first. But no one stepped forward until nearly 2000 years after Aristotle's death. In 1589, Galileo summoned learned professors to the base of the leaning Tower of Pisa. Then he went to the top and pushed off a ten-pound and a one-pound weight. Both landed at the same time. But the power of belief in the conventional wisdom was so strong that the professors denied what they had seen. They continued to say Aristotle was right.